CLAIM + DETAILED DESCRIPTION

[Claim(s)]

[Claim 1] The immunological measuring method characterized by contacting an immunological agglutination reaction reagent and analyte under existence of the heat denatured albumin of solution form voice in the immunological measuring method which the immunological agglutination reaction reagent and analyte which made the insoluble carrier support an antigen (or immune body) are contacted, and detects an immune body (or antigen).

[Claim 2] The immunological measuring method according to claim 1 whose insoluble carrier is a latex particle and whose antigen which this insoluble carrier is made to support is an antigen of TOREPANEMA PARIDAMU cell origin.

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the immunological measuring method using the immunological agglutination reaction reagent using an antigen-antibody reaction which can inhibit a nonspecific reaction effectively and simple and can perform it.

[0002]

[Description of the Prior Art] An immunological agglutination reaction reagent is a typical reagent using the agglutination reaction accompanying an antigen-antibody reaction. That is, with an immunological agglutination reaction reagent, the specific antigen (or immune body) is fixed by the insoluble carrier, and since condensation will take place by an antigen-antibody reaction if the immune body (or antigen) to the this fixed antigen (or immune body) exists, the above-mentioned immune body (or antigen) is detectable. What has singularity high as the antigen fixed by progress of antigen refining technology or an immune body comes to be obtained these days, and the application range in the clinical test of an immunological agglutination reaction reagent is expanded further.

[0003] As the typical example of the immunological measuring method using the above-mentioned immunological agglutination reaction reagent, The screening test of the syphilis which measures the antibody titer of the Treponema pallidum (Treponema Pallidum; it may omit Following TP) immune body in blood by immunological agglutination reaction using an antigen-antibody reaction etc. is mentioned. When

the reagent which the carrier was made to support with this method by using as an antigen ingredient what was solubilized after crushing the cell and cell of TP which were cultivated by the testis of the rabbit etc. catches the specific reaction by the antigen (TP antigen) and an immune body (TP immune body) as condensation of a carrier, TP immune body is detected.

[0004] However, in the above-mentioned method, in spite of TP immune body negativity, condensation of a reagent is caused by the reaction by ingredients other than TP immune body, i.e., a nonspecific reaction, a positivity (false positivity) may be shown, and it has been a problem by the sample to measure.

[0005] Various methods are examined in order to inhibit this nonspecific reaction. For example, the method of inhibiting a nonspecific reaction by using the water-soluble polymer which contains one or more glycoside inductors in JP,H4-122858,A by a monomeric unit as a condensation enhancer which promotes agglutination reaction is shown. Moreover, to JP,S58-144748,A, the method of adding bovine serum albumin (Bovine Serum Albumin; it omitting Following BSA) or horse serum albumin in a latex suspension is indicated. Moreover, to JP,S58-144748,A, adding the polypeptide of molecular weight 1000-10000 is indicated. Furthermore, the method of using for JP,H8-176195,A as a blocking agent what carried out chemical modification of the albumin with SH modification reagent after returning an S-S bond using a reducing agent under alkali conditions is indicated.

[0006]

[Problem(s) to be Solved by the Invention] Various methods are examined, in order that the false positivity generated by a nonspecific reaction may pose a big problem and may solve this problem in the immunological measuring method using immunological agglutination reaction so that it may see in the example of TP immune body measuring reagent as described above. However, there was a problem that the above methods of control of a nonspecific reaction were still insufficient, and the adjustment method of protein or a polypeptide to be used was complicated. Namely, this invention aims at developing a simple means to inhibit a nonspecific reaction effectively in the immunological measuring method which used the immunological agglutination reaction reagent.

[0007]

[Means for Solving the Problem] In order to solve the above-mentioned technical problem, as a result of trying hard wholeheartedly, this invention persons are the simple methods of making the albumin which heat-treats and carried out thermal denaturation exist in the system of reaction by solution form voice, find out that the above-mentioned nonspecific reaction can be inhibited effectively, and came to complete this invention.

[0008] Namely, this invention is set for the immunological measuring method which the immunological agglutination reaction reagent and analyte which made the insoluble carrier support an antigen (or immune body) are contacted, and detects an immune body (or antigen). It is the immunological measuring method characterized by contacting an immunological agglutination reaction reagent and analyte under existence of the heat denatured albumin of solution form voice.

[0009] According to the immunological measuring method of this invention, the nonspecific reaction used as causes, such as false positivity, can be inhibited simple, and reliable measurement is attained. By making the heat denatured albumin obtained by heat-treatment exist in the system of reaction, although the mechanism of action from which such an outstanding effect is acquired is not necessarily clear Since a certain interaction happens between the ingredient and heat denatured albumin which cause a nonspecific

reaction and a nonspecific reaction ingredient is masked, it is thought that nonspecific condensation is controlled.

[0010]

[Embodiment of the Invention] By the immunological measuring method of this invention, the immunological agglutination reaction reagent which made the insoluble carrier support an antigen (or immune body) is used. As an insoluble carrier used for this reagent, after supporting an antigen (or immune body), if an immune body (or antigen) and an antigen-antibody reaction are caused and condensed, it can be used, without restricting a well-known carrier in particular.

[0011] If the carrier which can be used conveniently is illustrated, polystyrene, a styrene methacrylic acid copolymer, A styrene glycidyl (meta) acrylate copolymer, a styrene styrene sulfonate copolymer, A methacrylic acid polymer, an acrylic acid polymer, an acrylonitrile styrene-butadiene rubber copolymer, The particulates of organic high polymers, such as RATTEKUSU, such as a vinyl chloride acrylic ester copolymer and polyvinyl acetate acrylate, Or silane coupling processing etc. is performed to an inorganic acid ghost like silica, silica alumina, and alumina, or this inorganic acid ghost, and particles of living thing origin, such as an inorganic particle which introduced the functional group, a Homo sapiens O type hemocyte, and a sheep red blood cell, etc. are mentioned. When RATTEKUSU particles are used as a carrier also in these carriers, an immunological agglutination reaction reagent suitable for automatic analysis is obtained.

[0012] Although the particle diameter in particular of the above-mentioned carrier is not limited, it is suitable to use the carrier whose mean particle size is 0.05-10 micrometers from viewpoints, such as the ease of happening of the condensation after an antigen-antibody reaction and the ease of carrying out of distinction of condensation.

[0013] The antigen (or immune body) supported with this invention by the above-mentioned insoluble carrier will not be limited especially if the immune body (or antigen) and antigen-antibody reaction which serve as a subject of examination, respectively are caused. If the antigen or immune body suitably used by this invention is illustrated, the antigen of TP cell ingredient origin for syphilis diagnosis, An anti human C reactivity protein (CRP) immune body besides the hepatitis B virus surface antigen (HBs) for hepatitis B diagnosis, an anti-alpha-fetoprotein (AFP) immune body, an anti-beta 2-microglobulin (beta2-m) immune body, etc. are mentioned.

[0014] An antigen (or immune body) can be used especially as a method which an insoluble carrier is made to support, without restricting a known method. As the fundamental support method, there are a physical adsorption method and the chemical joining-together method, and a physical adsorption process is suitably used in respect of the simple nature of support operation.

[0015] It is common for an insoluble carrier to be immersed in the dispersion liquid which distributed the antigen (or immune body) by a physical adsorption method, and to make the antigen in liquid (or immune body) stick to an insoluble carrier physically. [the solvent used at this time] although it will not be limited especially if it is the dissolution or the thing distributed uniformly, but a well-known solvent can use these substances that there is no restriction in any way In order to keep effective the physiological activity of the antigen (or immune body) to be used A physiological saline, Or it is suitable to use the phosphate buffer solution of about 200 mM, glycine buffer solution, Tris buffer of about 10-200 mM adjusted by pH 7-9, etc. from the buffer solution with which pH was adjusted, for example, 10mM adjusted by pH 6-8. Moreover,

although the antigen in the above-mentioned dispersion liquid or the concentration in particular of an immune body is not limited, it is suitable to make it distribute so that it may be set to 1 (a mug-antigen, or an immune body / ml liquid)-10 (a mg-antigen, or an immune body / ml-liquid) from support efficiency or a homogeneous viewpoint of support. The general conditions are as follows, although the conditions to which an insoluble carrier is immersed in these dispersion liquid, and an antigen (or immune body) is made to stick take support efficiency and operativity into consideration and should just determine them suitably for every kind of the kind and the immune body made to support of the insoluble carrier to be used, or antigen.

[0016] That is, it is suitable for the amount of the carrier used at the time of being immersed in these dispersion liquid to use at 0.001 to 15% (w/v) from a viewpoint of support efficiency or operativity, and it is [that what is necessary is for the kind of an immune body or antigen just to determine suitably] common to use it in the form of a suspension. Although what is necessary is just to choose suitably the temperature in which these dispersion liquid are made to immerse a carrier by the character of a carrier, or the ingredient of buffer solution, generally 4 degrees C - 50 degrees C are used suitably. As for time to immerse a carrier in these dispersion liquid, it is common to carry out 30 minutes - one whole day and night.

[0017] Thus, the carrier with which the antigen (or immune body) was supported is the purpose which prevents spontaneous agglutination, improves preservation stability or inhibits a nonspecific reaction, and it is common to perform blocking processing further. The method in particular of blocking is not restricted but can use a well-known method. To the antigen-antibody reaction made into the purpose, especially if the protein used for blocking is protein which can stick to an insoluble carrier with inactivity, it will not be limited, but generally bovine serum albumin, casein, etc. are used in respect of the ease of receiving, or economical efficiency. furthermore -- high -- denaturation protein like thermal denaturation bovine serum albumin is suitably used in that a sensitivity reagent can be adjusted.

[0018] After performing blocking processing, separation washing is carried out according to centrifugal separation etc., and the buffer solution which finally took into consideration an antigen-antibody reaction or the cohesiveness of particles, a keeping quality, etc., and was chosen suitably is distributed, and it is considered as an immunological agglutination reaction reagent.

[0019] [the immunological agglutination reaction reagent used in the immunological measuring method of this invention] It is a reagent using the phenomenon which a carrier condenses in connection with the antigen-antibody reaction which occurs when the immune body in analyte (or antigen) is contacted. The latex fixed quantity reagent with which a latex condensation reagent, a microtiter reagent, etc. measure the degree of condensation optically as a fixed quantity reagent again can be illustrated as a qualitative reagent. Moreover, an immunological agglutination reaction reagent is good also as a reagent form which consists of 1 liquid, and good also as a form of the 2 liquid type reagent used making the water-soluble medium adjusted separately distribute. In an immunological condensation reagent, you may add suitably sodium chloride for nonspecific reaction inhibitors, such as condensation enhancers, such as a polyethylene glycol, and bovine serum albumin, and salt concentration adjustment etc. As for these additive agents, in the case of a 2 liquid type reagent form, being added by the water-soluble above-mentioned medium is common. As this water-soluble medium, buffer solution, such as a phosphate buffer solution, glycine buffer solution, Tris buffer, and good buffer solution, is used suitably.

[0020] Especially if the immune body (or antigen) used as a measuring object is the solution which may have been dissolved or suspended as analyte used by the immunological measuring method of this

invention, it will not be limited, but it is common that it is of biogenic substance origin. As this biogenic substance, cell extract, such as sap, such as blood, urine, lymph, amniotic fluid, ****, and saliva, a blood vessel, internal organs, and the skin, etc. is mentioned, for example.

[0021] The method of contacting an immunological agglutination reaction reagent and analyte by this invention is performed by mixing an immunological agglutination reaction reagent and analyte. With the reagent of a 1 liquid type form, it is directly mixed with analyte, and, in the case of a 2 liquid type form, it is common that a reagent and analyte are mixed in the water-soluble above-mentioned medium.

[0022] In this invention, when contacting an immunological agglutination reaction reagent and analyte, it is characterized [greatest] by making thermal denaturation albumin of solution form voice live together.

[0023] The thermal denaturation albumin used by the immunological measuring method of this invention refers to the denaturation albumin which met [which met and polymer-quantified] by heat treatment. Although BSA, horse serum albumin, a human serum albumin, etc. are suitably used as albumin (henceforth raw material albumin) used as the raw material of heat denatured albumin, especially BSA is suitable in respect of the ease of receiving, or economical efficiency. Although the purity in particular of BSA is not limited, it is common to use the crystal article called Fraction V.

[0024] Heat treatment of raw material albumin is preferably performed in buffer solution among an aqueous solution. In particular the kind or pH of buffer solution are not limited, but what is necessary is just to choose them from well-known buffer solution in consideration of economical efficiency etc., if raw material albumin and albumin after heat treatment are the ranges which do not produce an insoluble sediment. If the buffer solution suitably used by this invention is illustrated, a phosphate buffer solution, glycine buffer solution, boric acid buffer solution, Tris buffer, good buffer solution, etc. will be mentioned. 10 - 200mM and the range of 4-11 are suitable for the concentration and pH of buffer solution.

[0025] When heat-treating raw material albumin and denaturing it, it is suitable to heat-treat 0.5 to 15% (w/v) of raw material albumin preferably 0.1 to 20% (w/v) from a viewpoint of denaturation efficiency. Here, especially if the temperature in the case of heat treatment is a temperature from which polymer quantification or a meeting of raw material albumin takes place, it will not be limited. Generally polymer quantification of raw material albumin or the degree of a meeting changes with the retention time at the temperature and this temperature at the time of heat treatment. When the temperature at the time of heat treatment is comparatively low, in order to acquire an effective heat-treatment object, it is necessary to lengthen retention time and, and when this temperature is high, it is necessary to shorten retention time. What is necessary is just to hold them at 35-70 degrees C suitably with the temperature of 30-80 degrees C generally for 1 to 24 hours for 0.5 to 48 hours, although what is necessary is just to determine suitably the temperature and the retention time at the time of heat treatment in consideration of operativity, efficiency, etc.

[0026] The heat denatured albumin obtained by having carried out the above heat treatments can check the degree of polymer quantification or a meeting by measuring native (raw material) albumin and mobility before heat treatment, for example by polyacrylamide gel electrophoresis. Moreover, the existence of the denaturation of albumin can also be checked by measuring the absorbance change in the wavelength of lambda= 280nm with a spectrophotometer.

[0027] Although the method in particular of contacting an immunological agglutination reaction reagent and analyte under existence of the heat denatured albumin of solution form voice by this invention is not limited,

after contacting analyte and heat denatured albumin beforehand in solution, it is suitable to make an immunological agglutination reaction reagent live together. Moreover, although the concentration of the heat denatured albumin in that case changes with measuring objects, it is suitable for it from the depressor effect of a nonspecific reaction, and a viewpoint of economical efficiency (prevention of superfluous use of heat denatured albumin) to consider it as 1 to 10% (w/v) as concentration in the solution with which an immunological agglutination reaction reagent and analyte live together.

[0028] Next, although the example using the latex fixed quantity reagent form which consists of 2 liquid of the following first agent and a second agent as an immunological agglutination reaction reagent is shown as an example of the immunological measuring method of this invention, this example does not limit this invention.

[0029] Shell agent: The following mixed liquor (1) buffer solution which uses (1), (2), and (3) as a basic ingredient 20 - 1000mM, Carrier which supported pH 4-12 (2) antigen (or immune body) 0.005 to 1.5% (w/v) (3) sodium chloride 50 - 300mM second agent : Following (4), (5) And mixed liquor (4) buffer solution which uses (6) as a basic ingredient 20 - 1000mM, pH 4-12 (5) sodium chloride 50 - 300mM (6) heat denatured albumin In addition, Tris buffer, a phosphate buffer solution, glycine buffer solution, boric acid buffer solution, or good buffer solution can be used as the above-mentioned buffer solution 1.5 to 15%.

[0030] The measuring method at the time of using the above-mentioned latex fixed quantity reagent form is as follows.

[0031] From 10 times, analyte is diluted with the second agent which contains thermal denaturation albumin first to about 30 times, and is gently put about 5 minutes by it. Subsequently, a shell agent is added, a suitable wavelength is chosen, the variation per hour of absorbance is measured, and the state of aggregation of a reagent is detected. Although the amount of the shell agent used changes with kinds of an immune body or antigen at this time, when the carrier concentration in the solution at the time of measurement considers it as 0.001 to 0.5%, measurement with high sensitivity and precision is performed.

[0032]

[Effect of the Invention] According to this invention, a nonspecific reaction can be inhibited by easy operation and it enables false positivity to perform immunological measurement reliable few very, using an immunological agglutination reaction reagent as a result.

[0033]

[Example] Hereafter, although a work example explains this invention in detail further, this invention is not limited to these work examples.

[0034] BSA(product made from Sigma, Fraction V)10g of preparation marketing of a work example 1 - 5(1) thermal-denaturation bovine-serum-albumin (thermal denaturation BSA) solution was dissolved in 100g of 50mM glycine buffer solution (pH 8.6). Subsequently, after shaking slowly in the temperature and time which are shown in Table 1, it cooled and saved at 4 degrees C.

[0035] (2) The antigen solubilized after crushing TP cell cultivated by the preparation rabbit testis of the shell agent (TP antigen support latex suspension) was diluted so that it might become in ml and 10microg /with 20mM glycine buffer solution (pH 8.6), and it was considered as TP antigen liquid. 0.1ml of polystyrene particle suspensions of the mean particle diameter of 0.3 micrometer and 5% of latex concentration were added to 0.9ml of the above-mentioned TP antigen liquid, and it mixed. After putting gently at 4 degrees C for 4 hours, it added and 2ml of aqueous solutions containing bovine serum albumin were put gently for

further 1.5 hours. subsequently, 2ml of 100mM sodium chloride, 0.1% sodium azide, and 0.1M Tris buffer of pH 8.0 which contains BSA 1% were added and suspended in ** (TP antigen support latex) obtained by centrifugal separation, and the shell agent was prepared.

[0036] (3) The thermal denaturation BSA solution used as the concentration shown in preparation 0.1M sodium chloride, 0.1% sodium azide, and Table 1 of the second agent (buffer solution) and 0.1M Tris buffer of pH 8.0 containing 1.5% of PEG-20000 were prepared, and it was considered as the second agent.

[0037] (4) An analyte Treponema pallidum hemagglutination (TPHA;Treponema Pallidum Hemagglutination) examination and fluorescence treponemal antibody absorption (FTA-ABS;fluorescent treponemal) From the serum with which it is checked by the antibody-absorption examination that it is TP immune body negativity, it is a nonspecific reaction with a latex fixed quantity reagent. Three samples of serum which is easy to raise was prepared.

[0038] (5) After carrying out addition churning of 10micro of the analytes I in a glass cell, it put on measuring method second agent 240microl gently for about 5 minutes at 37 degrees C. Subsequently, 80microl addition of the shell agent was carried out, it was agitated, the optical diameter variation in the wavelength of 700nm from after 30 seconds to 200 seconds was measured, and TP immune body concentration was calculated from optical diameter variation. Autoanalyzer TBA-30R type (made by Toshiba Medical Systems) was used for the above operation. The measurement result judged a positivity and under 20U as negativity for more than 20U. The result was shown in Table 1.

[0039]

[Table 1]

表 1

	BSAの加熱処理条件			non-SDS-PAGE	陰性検体測定結果					
	温度	時間	乙剤中	による分子量	検体A		検体B		検体C	
	(℃)	(h)	濃度	変化観察結果	濃度	判定	濃度	判定	濃度	判定
実施例1	37	4	2%	+	<10U	陰性	<10U	陰性	<10U	陰性
実施例2	37	20	2%	+	<10U	陰性	<10U	陰性	<10U	陰性
実施例3	50	4	2%	+	<10U	陰性	<10U	陰性	<10U	陰性
実施例4	70	1	2%	+	<10U	陰性	<10U	陰性	<10U	陰性
実施例5	37	4	5%	+	<10U	陰性	<10U	陰性	<10U	陰性
比較例1			0%		26U	陽性	21U	陽性	270U	陽性
比較例2	未処理	未処理	2%	−	<10U	陰性	30U	陽性	32U	陽性
比較例3	未処理	未処理	5%	−	<10U	陰性	52U	陽性	25U	陽性

陰性検体測定；非特異反応を起こしやすい個別検体を測定した。
＋；増加が明瞭と認められる　－；変化なし
２０U未満を陰性、２０U以上を陽性とした。

[0040] It carried out by the same operation as a work example 1 except not using preparation and the measuring method, and the quality assessment thermal denaturation BSA of a comparative example 1 (1) reagent and a sample. The result was shown in Table 1.

[0041] It carried out by the same operation as a work example 1 except having used non-denatured BSA instead of preparation and the measuring method of a comparative example 2-3 (1) reagent and a sample,

and the quality assessment thermal denaturation BSA. The result was shown in Table 1.

[0042] By using thermal denaturation BSA, a nonspecific reaction is inhibited and all three samples can be judged correctly to be negativity. By the system which does not use BSA, all three samples caused the nonspecific reaction and became a positivity (false positivity). Moreover, when Native BSA was used, the nonspecific reaction occurred by 2 in 3 samples sample.

[Translation done.]